

HNF1 α activates the aminopeptidase N promoter in intestinal (Caco-2) cells

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Abstract

The importance of HNF1 binding proteins for intestinal aminopeptidase N expression was investigated using the Caco-2 cell-line. Aminopeptidase N promoter activity in Caco-2 cells depends on the HNF1 element (positions –85 to –58) and co-transfection with an HNF1 α expression vector demonstrates a direct activation of the promoter by HNF1 α through this element. Electrophoretic mobility shift assays using nuclear extracts from Caco-2 cells show the presence of high amounts of HNF1 binding proteins irrespective of their state of differentiation.

Key words: Transcriptional regulation; Differentiation; Transcription factor dimerization; HNF1; Aminopeptidase N

1. Introduction

Aminopeptidase N (EC 3.4.11.2) (APN) is a digestive hydrolase expressed at high levels in the small intestine [1,2]. Two promoters separated by a large intron control the expression of the APN gene. The promoter immediately upstream of the coding region is active in cells from the liver and small intestinal epithelium (the epithelial promoter) whereas the other promoter (the myeloid promoter) is active in myeloid cells [3]. The liver transcription factor, HNF1 α , or related factors bind to the region –85 to –58 (formerly referred to as the LF-B1 element) in the APN epithelial promoter. The presence of the HNF1 site in the promoter is crucial for its function in liver cells as its removal results in 20 fold reduced promoter activity [4]. HNF1 α [5–7] and the closely related factor HNF-1 β [8–10] are homeodomain proteins of the POU class. They bind as homo and hetero dimers to a common HNF1 element found in many liver-specific promoters. HNF1 α and HNF1 β are expressed in enterocytes and various other epithelial cells [8,9] also expressing APN, suggesting a general importance of HNF1 binding proteins for epithelial expression of APN. In the present work we directly demonstrate the activation of the APN promoter by HNF1 α .

2. Materials and methods

2.1. Cell culture and transfection

Caco-2 and HeLa cells were grown as monolayers in minimum essen-

tial medium (Gibco BRL Life Technologies) supplemented with 10% fetal calf serum and 100 μ g/ml each of penicillin and streptomycin in a humidified atmosphere containing 5% CO₂. Subconfluent Caco-2 cells were transfected with either the set of promoter deletions described previously [4] (Table 1) or co-transfected with 5 μ g of the HNF1CAT reporter construct and increasing amounts of the HNF1 α expression vector RSVHNF1 [11] (Fig. 1B). 10 μ g of the β -galactosidase expression vector, PCH110 (Pharmacia, Uppsala, Sweden), was used as internal control. The calcium phosphate transfection procedure [12] was used with the modifications for adherent epithelial cells described in [13]. Cells were harvested 48 h after transfection. Measurements of β -galactosidase and chloramphenicol acetyl transferase activities were performed as described elsewhere [4]. For the deletion analysis a total of four independent experiments were carried out with each construct. HeLa cells were co-transfected by the standard CaPO₄ procedure with 5 μ g of APN1kCAT, HNF1CAT, –55CAT (referred to as AMPCAT, LF-B1CAT and the –55 deletion, respectively, in [4]) with or without addition of 5 μ g of the HNF1 α expression vector. 400 μ g of protein were used for the CAT assays and three independent experiments were performed.

2.2. Preparation of nuclear extracts and electrophoretic mobility shift assay (EMSA)

Nuclear extracts were prepared [14] from exponentially growing and post-confluent (12 days) Caco-2 cells and from pig small intestine [4]. EMSA [4] with a double-stranded oligonucleotide probe (APNHNF1) covering the APN promoter HNF1 site was carried out using either 8 μ g (Fig. 2A) or 1 μ g (Fig. 2B) of nuclear extracts [4]. For competition (Fig. 2B) the indicated molar excess of unlabelled APNHNF1 or ALBHNF1 (an oligonucleotide spanning the HNF1 site in the rat albumin promoter [15]) were used as specific competitors and the oligonucleotide, Sp1 [4], containing two Sp1 binding sites as the un-specific competitor.

3. Results

3.1. HNF1 α activates the APN promoter

A set of promoter deletions [4] placed in front of the bacterial gene for chloramphenicol acetyl transferase (CAT) was transfected into Caco-2 cells.

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Deletion of the promoter to position –123 had no effect on the activity but removal of the HNF1 site by deletion to position –55 reduced reporter gene expression 8-fold (Table 1). The activity was restored by insertion of the HNF1 site in front of the –55 deletion (Table 1; the HNF1CAT construct). Co-transfection with an HNF1 α expression vector [11] and APN promoter constructs into HeLa cells resulted in a 4- to 10-fold increase in the activity and this increase depended on the presence of the HNF1 region as it was not observed with the –55 deletion which contains the Sp1 site and the TATA box only (Fig. 1A). APN promoter activity was also stimulated when increasing amounts of the HNF1 α expression vector were co-transfected with the HNF1CAT reporter construct into Caco-2 cells (Fig. 1B)

3.2. HNF1 binding dimers differ between small intestinal mucosa and Caco-2 cells

Nuclear extracts were prepared from pig small intestinal mucosa, exponentially growing and post-confluent Caco-2 cells. EMSA was performed with these nuclear extracts and a double-stranded probe covering the HNF1 site in the APN promoter (APNHNF1). As seen in Fig. 2A all three extracts generated a retarded band with low mobility. The retarded bands obtained with the Caco-2 extracts migrated with the same mobility whereas the band obtained with the small intestinal extract had

a slightly higher mobility. The major retarded band generated with the Caco-2 nuclear extracts could be competed out using the unlabelled double-stranded oligonucleotides, APNHNF1 and ALBHNF1 (an oligonucleotide spanning the HNF1 site in the rat albumin promoter [15]), but not with an oligonucleotide containing Sp1 binding sites (Fig. 2B). In addition the Caco-2 extracts generated a second distinct band (Fig. 2A, marked with an asterisk). This band is not affected by the addition of the competitors (Fig. 2B; the band is faint due to the low amounts of nuclear extracts used) and we consider it to be generated by an unspecific DNA binding activity present in the Caco-2 nuclear extracts.

4. Discussion

The deletion analysis of the APN epithelial promoter demonstrates the importance of the HNF1 site for its function in intestinal cells as removal of the site resulted in an 8-fold reduction in promoter activity. Furthermore no other element between the HNF1 site and position –1,182 had any effect on the promoter activity in Caco-2 cells. The identification of the region –85 to –55 in the APN epithelial promoter as an HNF1 site was previously based on indirect evidence [4]. Here we present direct evidence for the activation of the APN epithelial pro-

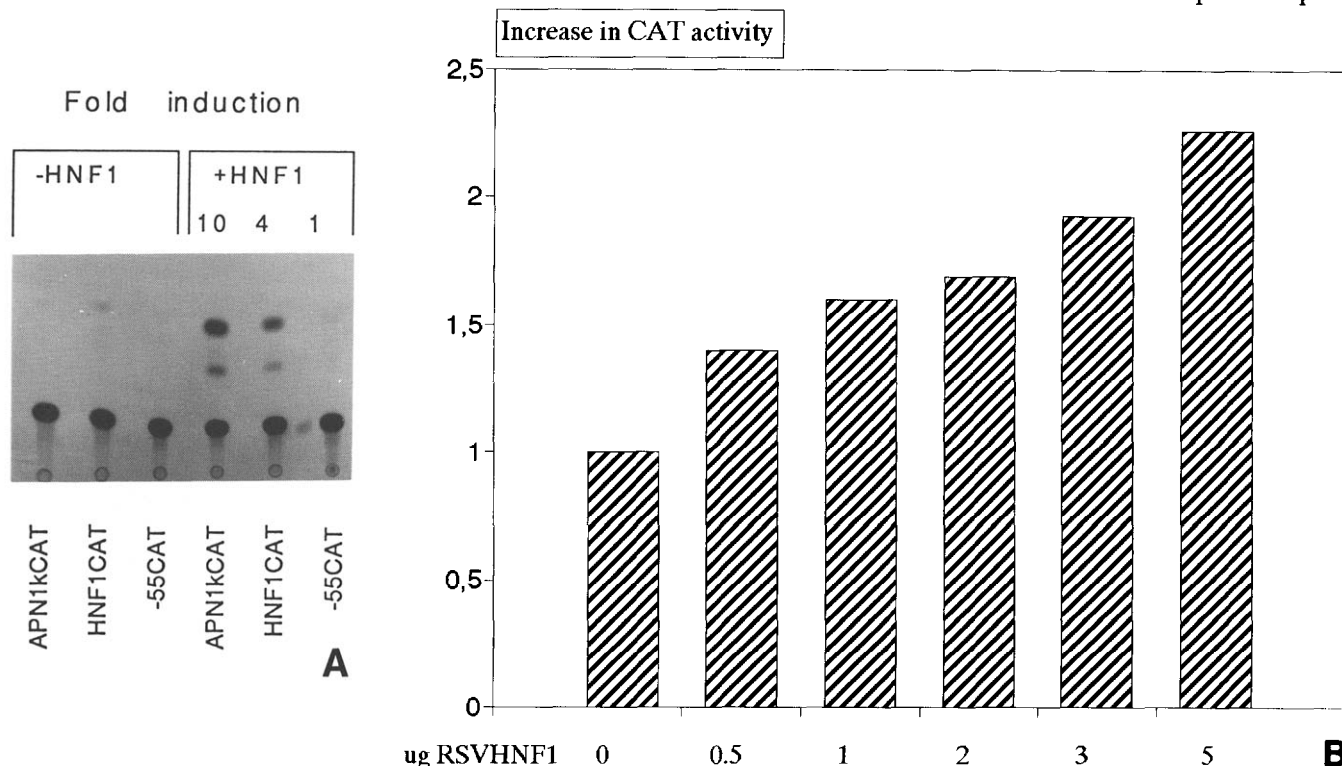


Fig. 1. HNF1 α activates the APN epithelial promoter. (A) Activation in HeLa cells. Co-transfection of APN promoter constructs and the HNF-1 α expression vector into HeLa cells results in a stimulation of reporter gene (CAT) expression. The induction of CAT expression depends on the presence of the HNF-1 site as the –55 deletion lacking this element is not affected by the expression of HNF-1 α . (B) Activation in Caco-2 cells. Co-transfection of increasing amounts of the HNF1 α expression vector into Caco-2 cells results in increased APN epithelial promoter-driven reporter gene expression. 5 μ g of the HNF1CAT reporter plasmid was used and the activity is expressed relative to the activity observed with this construct alone.

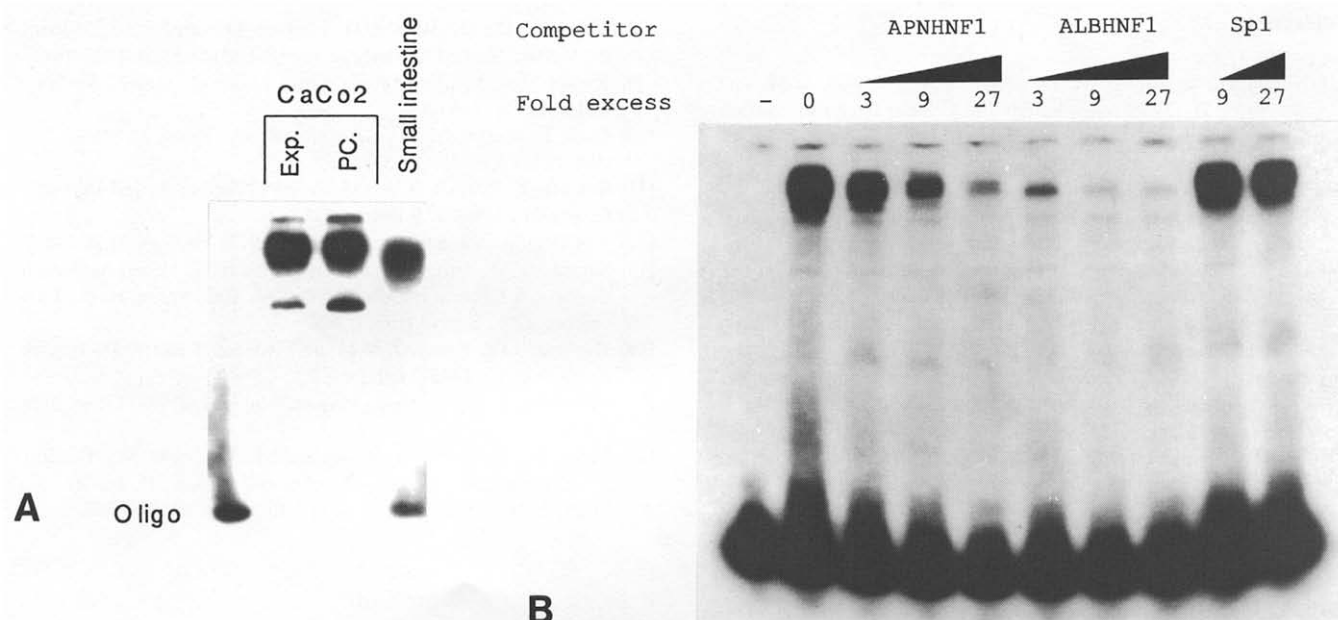


Fig. 2. EMSA with CaCo-2 and small intestinal nuclear extracts. (A) The composition of HNF1 binding dimers differs between nuclear extracts from the small intestine and CaCo-2 cells. EMSA was carried out with a double-stranded oligonucleotide (APNHNF1) covering the HNF1 site in the APN epithelial promoter and nuclear extracts prepared from small intestinal mucosa, exponentially growing (Exp.) and 7 days post-confluent (PC.) CaCo-2 cells. (B) The major retarded band is due to specific protein–DNA interactions. EMSA was performed with the APNHNF1 probe and 1 μ g of CaCo-2 nuclear extracts prepared from post-confluent CaCo-2 cells. Increasing amounts of unlabelled competitor were included in the binding reactions. Either APNHNF1 or ALBHNF1 (an oligonucleotide spanning the HNF1 site in the rat albumin promoter) were used as specific competitors. An oligonucleotide containing Sp1 binding sites was used as the unspecific competitor.

motor by HNF1 α . The activation by HNF1 α depends on the presence of the HNF1 site and occurs in both CaCo-2 cells and in HeLa cells. The latter expresses only low levels of HNF1 binding proteins [8] and APN mRNA. Post-confluent CaCo-2 cells are known to contain higher levels of aminopeptidase N activity compared to exponentially growing CaCo-2 cells [16].

Table 1
Deletional analysis of the APN promoter in CaCo-2 cells

Construct	% of pAPN1kCAT
-1,122	100
-788	127
-603	118
-360	73
-215	87
-177	68
-123	87
-55	12
HNF1CAT	87
UFCAT	14

Deletions (end points indicated) of the APN promoter placed in front of the CAT gene were transfected into CaCo-2 cells. CAT activity is expressed as a percentage of the activity obtained using the pAPN1kCAT construct which contains 1,122 bp of the 5' upstream region. The -55 deletion contains the Sp1 site (nucleotides -53 to -30) and the TATA box (nucleotides -29 to -24) from the APN promoter. The constructs HNF1CAT and UFCAT contain the HNF1 region (nucleotides -85 to -58) and the UF region (nucleotides -112 to -90), respectively, in front of the -55 deletion.

Exponentially growing as well as post-confluent CaCo-2 cells contained similar levels of HNF1 binding activity and no difference in the composition of the HNF1 binding dimers was observed in EMSA (Fig. 2A), suggesting that the increase in APN expression in post-confluent CaCo-2 cells is not mediated by the HNF1 site. HNF1 α homodimers migrate with lower mobility in EMSA compared to HNF1 β homodimers. HNF1 α /HNF1 β heterodimers migrate with intermediate mobility [8,9]. Thus the observed difference in EMSA with CaCo-2 and small intestinal nuclear extracts might reflect a difference in the composition of the HNF1 dimers binding to the APN HNF1 site. HNF1 α is expressed at higher levels than HNF1 β in both enterocytes and CaCo-2 cells [8,9]. The observed difference in the EMSA suggest, however, that the formation of the larger HNF1 α homodimers are favored in CaCo-2 cells and the smaller HNF1 α /HNF1 β heterodimers in the enterocyte. Alternatively the difference could be due to either proteolytical degradation of the HNF1 proteins in the small intestinal nuclear extract or a species difference in the sizes of the HNF1 proteins.

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